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The nature, role and control of lithobionts on stone cultural heritage: weighing-up and managing biodeterioration and bioprotection

Sergio Enrico Favero-Longo^{1,*} & Heather A Viles²

¹Università degli Studi di Torino
Dipartimento di Scienze della Vita e Biologia dei Sistemi
Viale Mattioli 25, 10125 Torino, Italy
Tel. +390116705972
Fax +390116705962
sergio.favero@unito.it
orcid.org/0000-0001-7129-5975

²University of Oxford,
School of Geography and the Environment
South Parks Road, Oxford, OX1 3QY, Oxford, UK
Tel: +44 1865 285070
heather.viles@ouce.ox.ac.uk
orcid.org/0000-0002-2444-1295

*corresponding author: sergio.favero@unito.it

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35 **Abstract**

36 Lithobionts (rock-dwelling organisms) have been recognized as agents of aesthetic and physico-
37 chemical deterioration of stonework, and removal of microorganisms from cultural heritage stone
38 surfaces (CHSS) is widely considered a necessary step in conservation interventions. On the other
39 hand, lithobiontic communities can help integrate CHSS with their environmental setting and
40 enhance biodiversity. Moreover, in some cases bioprotective effects have been reported and even
41 interpreted as potential biotechnological solutions for conservation.

42 This paper reviews the plethora of traditional and innovative methodologies to characterize
43 lithobionts on CHSS in terms of biodiversity, interaction with the stone substrate and impacts on
44 durability. In order to develop the best management and conservation strategies for CHSS, such
45 diagnosis should be acquired on a case-by-case basis, as generalized approaches are unlikely to be
46 suitable for all lithobionts, lithologies, environmental and cultural contexts or types of stonework.

47 Strategies to control biodeteriogenic lithobionts on CHSS should similarly be based on
48 experimental evaluation of their efficacy, including long term monitoring of their effects on
49 bioreceptivity. This review examines what is known about the efficacy of control methods based on
50 traditional-commercial biocides, as well as those based on innovative application of substances of
51 plant and microbial origin, and physical techniques. A framework for providing a balanced
52 scientific assessment of the role of lithobionts on CHSS and integrating this knowledge into
53 management and conservation decision-making is presented.

54

55 **Keywords:** biocide, biodiversity, biofilm, bioreceptivity, conservation of cultural heritage stone
56 surfaces, stone cleaning, stone durability

57 **The issue of lithobionts on the stone cultural heritage: an introduction**

58 The preservation and transmission to future generations of stone cultural heritage (objects, buildings
59 and sites carved in, or built from, stone), including rock art, archaeological and historical
60 monuments and artistic stonework, are globally shared and signed duties, which need to be
61 combined with the protection and conservation of natural heritage (Convention Concerning the
62 Protection of the World Cultural and Natural Heritage 1972). As with other global issues in today's
63 changing world, scientific advances can inform effective and environmentally conscious
64 management, based on correct identification of threats, and development of sustainable solutions
65 (Ferretti and Comino 2015; Charter and Tischner 2017). The growth of lithobionts (i.e. rock-
66 dwelling organisms) on cultural heritage stone surfaces (CHSS; Fig. 1) has long been associated
67 with biodeterioration, which can be defined as any undesirable change in the properties of a
68 material caused by the vital activities of organisms (Hueck 1965), and recognized as a threat to
69 conservation (Caneva et al. 2008). This is not surprising given widespread evidence of
70 biogeophysical processes (such as mechanical fracturing and disruption of rocks and minerals by
71 living structures) and biogeochemical processes (chemical destabilization and compositional change
72 of rocks and minerals by metabolic processes and products), which are vital to pedogenesis
73 (Silverman 1979; Totsche et al. 2010). Plenty of field and experimental data demonstrate rock
74 weathering by macro- and micro-organisms (bioweathering), such as vascular plants (Pawlik et al.
75 2016) and bryophytes (Ricci and Altieri 2008), lichenized- (Seaward 2015) and non-lichenized
76 fungi (Gadd 2017), photo- (Albertano 2012) and chemo- lithotrophic bacteria (Mapelli et al. 2012).
77 Coupled with the desire to remove lithobionts from heritage stone surfaces for aesthetic reasons
78 (because they can produce unsightly discoloration and obscure important carved details), such
79 biodeteriorative roles strengthen the case for conservation interventions (Pinna 2017).

80 On the other hand, lithobiontic communities have also been seen as enhancing biodiversity, and in
81 some cases bringing positive aesthetic characteristics to CHSS (see section *Factors affecting the*
82 *opportunity to remove (or preserve) lithobionts*). Furthermore, the last two decades have seen an

83 increasing interest in whether lithobiontic communities can in some circumstances also act as a
84 bioprotective layer, covering stone surfaces and limiting weathering processes driven by abiotic
85 factors, such as meteorological forces and air pollutants (Carter and Viles 2005; McIlroy de la Rosa
86 et al. 2012). Such findings, mixed with advances in investigating biomediated approaches
87 (microbial biocementation) which can promote the consolidation of stone (Fernandes 2006; Wang
88 et al. 2016; Shraddha and Darshan 2019), have contributed to the recent proposal that bioprotection
89 of buildings and cultural heritage by lithobionts may be a sustainable strategy (Gadd and Dyer
90 2017). It is important not to see contrasting research findings on biodeterioration and bioprotection
91 as conflicting positions, as they likely represent different aspects of complex interactions within and
92 between natural or cultural heritage ecosystems (see section *Biodeteriorative and/or bioprotective*
93 *effects*). Misunderstandings on this point may indeed critically impact decisions on the management
94 of lithobionts on CHSS, which should not be based on generalized views of biodeterioration vs
95 bioprotection as ideologically opposed ‘schools of thought’, but on diagnostic analyses targeting
96 each case. Advances in molecular, microscopy and spectroscopy methods since the 1990s have
97 strongly improved the likelihood of characterizing the diversity of lithobionts on CHSS, and their
98 related biogeophysical and biogeochemical processes (Piñar and Sterflinger 2018; Sanmartín et al.
99 2018; Schröder et al. 2019). In the following section, colonization patterns and physico-chemical
100 processes driven by the major groups of lithobionts are exemplified, together with a critical
101 evaluation of what diagnostic approach is required to reliably unveil their biodeterioration and/or
102 bioprotection potential on CHSS. In the second part, approaches to manage lithobionts on CHSS are
103 outlined, and a potential decision framework based on experimental assessment and monitoring of
104 both the roles of lithobionts and the efficacy of control strategies is introduced.

105

106

107

108 **Patterns and impact of lithobiontic colonization**

109 *Stone bioreceptivity and biogeophysical and biogeochemical impacts of lithobionts*

110 Natural rock surfaces and CHSS provide interfaces between geological materials, air and water
111 which are invariably colonised by lithobionts (Gorbushina and Broughton 2009). Intrinsic physico-
112 chemical properties of stone materials (e.g. surface roughness, porosity, mineral, composition) are
113 the primary factors determining their bioreceptivity, i.e. their aptitude to be colonized by living
114 organisms (Guillitte 1995; Miller et al. 2010). The rate of supply of biological particles, usually
115 resistant structures (spores, pollens) dispersed as bioaerosol, is the main complementary factor
116 needed to start and support colonization dynamics (Mandrioli et al. 2003). Other extrinsic factors,
117 such as climatic conditions and the availability of organic nutrients, influence the composition and
118 structure of lithobiontic communities on CHSS, with their network of nutritional interactions
119 (autotrophs/producers; heterotrophs/destroyers and consumers) (Caneva et al. 2008).

120 An index to quantify bioreceptivity has been proposed to evaluate the performance of any
121 construction material and support management decisions in the ornamental stone industry which
122 can be applied to CHSS (Vázquez-Nion et al. 2018). In the suggested protocol, the growth of a
123 standard photoautotrophic community (bryophyta, green algae, cyanobacteria) on coupons of
124 different lithologies is compared by fluorimetry (to quantify the amount of chlorophyll *a*) and
125 spectrophotometry (to quantify colour change. Such an approach reflects the fact that aesthetic
126 appearance (colour, visibility of artistic details) is important for perceptions of the value of CHSS
127 (Brimblecombe and Grossi 2005). On the other hand, such an index does not take account of the
128 three-dimensional phenomenon of lithobiontic colonization of CHSS, in which the hidden
129 dimension, below the surface, represents the major interface between biotic and mineral
130 components.

131 Across a range of spatial scales, higher plants, bryophytes, lichens and microorganisms anchor or
132 adhere to mineral substrates, including CHSS, in order to provide stability, exploit water and/or
133 nutrients, and as a consequence modify the physico-chemical properties of the substrates. The root

134 systems of woody plants growing directly on pavements or masonries, and, in the case of tree, in
135 proximity to building foundations and hypogean structures, can exert mechanical forces and cause
136 superficial and structural damage, especially in archaeological sites (Caneva et al. 2009; Bartoli et
137 al. 2017). The penetration of slender rhizoids and protonemata of mosses (even though not enforced
138 by lignin) have been related to mechanical damage of mosaics and wall paintings (Saiz-Jimenez et
139 al. 1991; Ricci and Altieri 2008). In the case of lichens (Fig. 2 a-d), the penetration of mycobiont
140 hyphae beneath epilithic crustose thalli, and of the anchoring points of rhizinae and haptera of
141 foliose and fruticose lichens, respectively, has been microscopically characterized within many
142 different stone materials (de los Ríos and Ascaso 2005; Salvadori and Casanova-Municchia 2016).
143 Different patterns of penetration are found depending on the mineralogical and microstructural
144 features of different lithologies, and the species involved, with hyphal organization, spread, and
145 depths ranging from a few microns to several millimeters (Favero-Longo et al. 2005; Scarciglia et
146 al. 2012; Sohrabi et al. 2017). Moreover, some lichen species display an endolithic habit, with the
147 thallus (including the photobiont layer) growing entirely within the rock substrate, exploiting
148 internal cracks (chasmo-endolithic) or intrinsic porosities (crypto-endolithic), or actively dissolving
149 minerals (eu-endolithic) (Pinna et al. 1998; Casanova-Municchia et al. 2014; Favero-Longo et al.
150 2015).

151 Similar ranges of growth, from fully epilithic to fully endolithic, also characterize autotrophic and
152 heterotrophic microorganisms usually organized as biofilms, which are adapted to every kind and
153 level of environmental stress in terms of temperature, water and nutrient availability, and solar
154 irradiation (Gorbushina and Broughton 2009). Archaea, bacteria and eukaryotic microbes, as non-
155 lichenized fungi, widely live embedded in an extracellular matrix of biopolymers, usually known as
156 as EPS (Extracellular Polymeric Substances), which is dominated by polysaccharides, but also
157 contains (glyco-)proteins, glycolipids and DNA (Flemming et al. 2007, 2017). High hydration of
158 EPS makes biofilms functionally active and resistant environments, favouring the nutrition,
159 communication and defence of microbes, and also drives their physico-chemical interactions with

160 the substrate. Cyclic hydration and dehydration of biofilms cause volume changes and, as a
161 consequence, pressures at their points of adhesion, causing disaggregation and detachments (Negi
162 and Sarethy 2019). A similar mechanical action is exerted by mosses and lichens, whose water
163 status varies passively with environmental conditions (poikilohydric organisms; Seaward 2015).

164 The covering of rock surfaces and CHSS by coloured dark patinas and thalli also influences the
165 thermal behaviour of the surface and consequent physical stress (Garty 1990; Carter and Viles
166 2004). In parallel, water availability at the lithobiont-rock interface favours the mobilization of
167 metabolites and mineral ions, supporting chemical modification and sometimes dissolution of the
168 original mineral constituents and the precipitation of new (bio-)minerals (Banfield et al. 1999).

169 Accordingly, protons excreted by plant root tips induce cationic exchange and contribute to ion
170 mobilization from the contacted minerals, and their chemical modification (Caneva et al. 2008).

171 Bryophytes, like algae and cyanobacteria, can favour the precipitation of carbonates, as a
172 consequence of photosynthetic removal of CO₂ and their ability to solubilize and bind Ca, a
173 phenomenon reported for natural springs, but also for fountains and monuments in humid regions
174 (Bolívar and Sánchez-Castillo 1997; Ortega-Morales et al. 2000; Crispim and Gaylarde 2005; Ricci
175 and Altieri 2008). Several lichenized and non-lichenized fungi secrete a large variety of primary
176 and secondary metabolites with acidic and chelating functions, supporting acidolysis and
177 complexolysis (Piervittori et al. 2009; Gadd 2017). The biomineralization at the lichen-substrate
178 interface of different oxalates (e.g. Ca-, Mg-, Fe-, Mn-oxalate), depending on the rock metal
179 contents, has frequently been cited as evidence of the direct influence of the mycobiont secretion of
180 oxalic acid on colonized surfaces (Adamo and Violante 2000; Chen et al. 2000; Gadd et al. 2014).

181 In particular, calcium oxalates (whewellite and weddellite) have been reported on a wide range of
182 lithologies, even those poor in Ca, because of their very low solubility in comparison with other
183 oxalate species (Fig. 2 e-f; Favero-Longo et al. 2005), and they are often found on CHSS colonized
184 by certain lichens (Edwards et al. 2003; Pena-Pozo et al. 2018; Tonon et al. 2019). The insolubility
185 of Ca-oxalates also accounts for their long persistence on CHSS, where their biological origin has

186 been suggested as an alternative to a chemical origin (e.g. due to degradation of restoration
187 products) even in the absence of viable lithobiontic communities (Caneva et al. 1993). Mineral
188 leaching by other fungal primary metabolites (e.g. citric and malic acids) has also been documented
189 (Wei et al. 2012; Sazanova et al. 2016), but they have generally received less attention in the field
190 of CHSS in comparison to oxalates because of the absence of clear or persistent traces. Mineral
191 leaching activity by complexation has also been reported for some depsides, depsidones and
192 pulvinic acid derivatives (secondary metabolites exclusive to lichens) although their low solubility
193 may limit their impact (Ascaso et al. 1976; Haas and Purvis 2006; Favero-Longo et al. 2013). All
194 these metabolites, as well as the involvement of EPS, seem to contribute to the weathering of
195 silicate minerals into clays, as TEM investigations have showed for the lichen-driven
196 vermiculization of biotite and other cases (Cuadros 2017), and in several cases to the dissolution of
197 carbonates (Pinheiro et al. 2019).

198 Different processes need to be invoked to explain the euendolithic behaviour of certain lichen
199 species, as they do not secrete either oxalic acid or the above mentioned lichen secondary
200 metabolites (Pinna et al. 1998). In some cases respiration-induced acidification of the substrate has
201 been shown to be sufficient to provoke pitting activity (formation of sub-millimetric cavities on the
202 surface) (Weber et al. 2011), but the involvement of siderophore-like compounds has also been
203 hypothesized, as these complexing compounds, involved in iron nutrition, also scavenge calcium if
204 iron availability is low (Favero-Longo et al. 2011). Similarly to endolithic lichens, microcolonial
205 fungi (MCF), which are a group of fungi tolerant of the extreme stress of exposed substrates and
206 thus colonizing bare rock surfaces, including CHSS (Sterflinger 2010), are responsible for
207 dissolution and pitting phenomena on carbonate and silicate rocks, but the processes responsible
208 still need to be clarified (De Leo et al. 2019). The rigidity of their cell wall due to melanin
209 deposition has been related to their penetration ability (Sterflinger and Kumbein 1997), but ion
210 mobilization by chelating molecules (Favero-Longo et al. 2011) and/or corrosive EPS containing
211 pullulan and galactofuromannan as the main constituents (Breitenbach et al. 2018) may be involved.

212 The strong negative charge of cyanobacterial EPS, due to uronic acids and sulphate groups, also
213 contributes to the dissolution of cations from colonized substrata and their microbial adsorption
214 (Bellezza et al. 2006; Albertano 2012). Sulfuric, nitrous and nitric acids are released by
215 chemolithotrophic (sulphur-oxidizing, nitrifying) bacteria and (ammonia-oxidizing) archaea,
216 through enzyme catalyzed oxidation of inorganic compounds, such as ammonia, elemental sulphur
217 and hydrogen sulphide, and are likely to have impacts on CHSS (Warscheid and Braams 2000;
218 Zhang et al. 2019).

219

220 *Biodeteriorative and/or bioprotective effects*

221 All the above-mentioned (and many other) patterns of lithobiont-substrate interactions, in terms of
222 biogeophysical and biogeochemical processes, have been increasingly detailed in the last two-three
223 decades and supported by a huge number of study cases, including CHSS. This has certainly helped
224 clarify the worldwide distribution and impacts of certain phenomena, but, in some cases,
225 experimental evidence obtained by advanced, 21st century technologies simply confirms early
226 insights dating back to the first part of the 20th century and before. For example, early descriptions
227 and accurate microscopic observations of lichen growth within rocks (Gümbel, 1856; Fry 1924
228 1927), as well as the recognition of lichen-driven chemical processes due to oxalic acid secretion
229 and/or a respiration-induced acidification (Slater, 1856; Uloth 1861; Sollas, 1880; Smith 1921),
230 provided convincing evidence of lichen biogeophysical and biogeochemical processes and are still
231 widely quoted today. However, the advent in recent years of a wide range of portable, non-
232 destructive methods now permits sophisticated measurements (such as spectroscopic analysis)
233 which can characterize the presence of metabolites and biominerals on/within CHSS in situ
234 (Maguregui et al. 2012; Costantini et al. 2018). Advances in molecular biology, and in particular the
235 diagnostic power of next-generation sequencing, have highlighted the huge variety of
236 microorganisms which can occur together on and beneath rock surfaces and CHSS, only partially

237 detectable by microscopy and culturing approaches, and not always directly related to more visible
238 surface colonizers (Bjelland et al. 2011; Piñar and Sterflinger 2018; Trovão et al. 2019).

239 On the other hand, whilst the observation and detection of (micro-)organisms, their structures and
240 metabolites, or their molecular signs, certainly implies present or past interactions with rock
241 substrates and CHSS, it does not necessarily mean that they play a biodeteriorative role as is often
242 routinely thought. In this context, DNA-metabarcoding analyses particularly need careful evaluation
243 to decipher which microorganisms are growing on and actively interacting with the substrates, and
244 which simply occur as past traces, dormant structures or episodic (dust) contaminants (Marvasi et
245 al. 2019). Implementation of molecular analysis pipelines, including the storage and effective
246 access of datasets dedicated to the biodeterioration research area, also represent an ongoing
247 challenge (Sterflinger et al. 2018).

248 Whilst there is incontrovertible evidence of mineral disintegration, chemical modification and
249 leaching by lithobionts, other studies have highlighted bioprotective activities, which were also
250 hypothesized many years ago (Krumbein 1968). Such hypotheses were based on early macroscopic
251 observations of differential erosion rates with and without lithobionts, suggesting an umbrella-like
252 protective effect (Mottershead and Lucas 1990; Özvan et al. 2015). Such interpretations of
253 lithobionts as forming a physical barrier against other weathering factors have been supported by
254 the quantification of lower solutional weathering from lithobiont (lichen)-covered limestone slates
255 in comparison with uncolonized controls after 1 year of exposure in the humid climate of Ireland
256 (McIlroy de la Rosa 2014). Ivy (*Hedera helix*) has been found to also have an umbrella-like impact
257 and protect historic limestone walls from pollutants (which can lead to soiling and deterioration)
258 (Sternberg et al. 2011). Additionally, experimental studies illustrate that *Hedera helix* can also
259 protect vulnerable historic stone walls from freeze-thaw damage through modifying microclimatic
260 conditions (Coombes et al. 2018). In drier climates, rock surface microorganisms have also been
261 demonstrated to be chemically involved in the development of rock coatings, which stabilize the
262 rock surfaces and contribute to their long term preservation (Taylor-George et al. 1983; Dorn 2013).

263 Nanometer-scale transport of Fe and Mn accumulated in bacterial sheaths and fixed on clay
 264 minerals contributes to the polygenetic formation of rock varnish (Dorn 2013). In arid Jordan,
 265 biofilms of cyanobacteria and fungi have been shown to contribute to case-hardening of sandstone
 266 by aiding cementation (Viles and Goudie 2004), and in cold arid central Antarctica, endolithic
 267 lichens and their EPS have been found to biomineralize iron oxides (Guglielmin et al. 2011). Such
 268 effects are also found in wetter climates, for example in the UK where the deposition by epilithic
 269 lichens of silica-rich layers within cracks and along mineral boundaries has been observed on
 270 granite outcrops alongside biophysical and biochemical weathering impacts (Lee and Parsons
 271 1999). Biomineralization of oxalates has also been considered to produce a potential protective
 272 shield against abiotic weathering agents, such as wind and runoff (Souza-Egipsy et al. 2004), and
 273 Ca-oxalates have often been proposed as producing potentially protective coatings for CHSS (e.g.
 274 Rampazzi 2019).

275 Multiple biodeteriorative and bioprotective roles of lithobionts have often been proposed. A notable
 276 example is the analysis of roles of the lichen *Verrucaria rubrocincta* on caliche plates in the
 277 Sonoran desert (Bungartz et al. 2004; Garvie et al. 2008): simultaneous biodeterioration of the rock
 278 substrate and a counterbalancing biomineralization of a upper protecting layer of fine-grained
 279 micrite have been recognized and characterized by isotopic analyses. Similarly, calcite dissolution
 280 and biomineralization of neoformed calcite around hyphae have been described for non-lichenized
 281 fungi (Fomina et al. 2010). Such recognition of both deteriorative and protective effects of
 282 lithobionts, and a deep evaluation of the prevailing process, may become crucial in future studies of
 283 lithobionts on CHSS (Bartoli et al. 2014). Observations of lithobiont (lichen) communities on tuff
 284 churches in Cappadocia showed the deteriogenic activity of penetrating hyphae, whilst some
 285 bioprotective effects were simultaneously envisaged, leaving uncertainty about whether or not the
 286 growths should be removed (Casanova-Municchia et al. 2018). Such a case study exemplifies the
 287 need for case-specific information on the multiple interactions of lithobionts and CHSS, their spatial
 288 patterning and their persistence over time, but also on their actual influence on stone durability,

289 before appropriate management decisions can be made. Indices to evaluate and quantify the
290 potential biodeteriogenic impact of plants (Signorini 1996), lichens (Gazzano et al. 2009) and
291 microorganisms (Gazzano et al. 2011) have been proposed, but are more oriented to a comparative
292 evaluation of the degree of physico-chemical interaction of different species with the substrate,
293 rather than to quantitatively evaluate their impact on the CHSS (Pinna 2014). As a framework
294 proposal, Table 1 summarizes a range of biodeteriorative and bioprotective roles reported for
295 various types of lithobiontic organisms. It is worth remarking that each (potential) role has to be
296 considered species-specific and cannot be generalized for all the members of each group; moreover
297 it also depends on the lithology and the environmental conditions (see the next sub-section).

298

299 *Lithobionts and stone durability*

300 Biodeteriorative and bioprotective processes may or may not balance out, and thus produce
301 complex impacts on the intrinsic stone properties relevant for durability, which can be defined as
302 the capacity of a building material to maintain its size, shape, strength and aesthetic appearance
303 over time (Bell 1993). In particular, knowledge of lithobiont-rock interactions should be combined
304 with quantitative investigation of their impacts on petrophysical properties, including porosity,
305 presence of swelling clay minerals, compactness, water absorption, presence and distribution of
306 anisotropies, compressive strength, and surface hardness (Molina et al. 2013; Wilhelm et al. 2016).
307 Relatively few studies have yet been made of the impacts of lithobionts on durability and resilience
308 of CHSS, although some research has focused on (bio-)geomorphological studies of natural rock
309 outcrops (Viles 2019). The results seem to depend strongly on lithology. For example, lichen
310 colonization has been found to correlate with surface hardening and a reduction of water absorption
311 on tuff (Garcia-Vallès et al. 2003), with a reduction of surface hardness on gneiss (McCarroll and
312 Viles 1995; Favero-Longo et al. 2015), and with variable patterns of surface hardening or softening,
313 and increased water absorption after the scraping of thalli from the surface on limestone (Morando
314 et al. 2017). Such variability suggests the need for tailored investigations to cover a range of

315 lithologies, different lichen species, and other, even more overlooked, lithobionts. Experimental
316 datasets are needed to test conceptual models on the balance between biodeterioration and
317 bioprotective processes and the net impact of lithobionts (lichens, in particular) on stone surfaces
318 (McIlroy de la Rosa et al. 2012). In this regard, one particularly important future research direction
319 is elucidating the impact of lithobionts on their substrate beyond their life-span. Surfaces once
320 covered by lithobionts which have been removed by conservation interventions, or have simply
321 died, may indeed display a long term protection effect due to past biogeochemical processes (e.g.
322 protection by a biomineral deposit, cementation, pore-filling by biogenic silica or varnish).
323 Conversely, such surfaces might also be more prone to disintegration following the loss of
324 biological structures which bound the disentangled mineral fragments. The latter scenario appears
325 particularly critical where stone surface details are a crucial component of the heritage values, as in
326 the case of carved rock-art (Tratebas 2004; Favero-Longo et al. 2019).

327 Developments in laboratory experiments using cultured model lithobiontic organisms, genetically
328 modified lineages, and the combination of closed and open experimental systems provide useful
329 new approaches to disentangling the impacts of lithobionts on CHSS. For example, several studies
330 have cultivated lithobionts on stone coupons and evaluated their impact on stone properties relevant
331 for durability (Favero-Longo et al. 2009; Villa et al. 2015; Pokharel et al. 2017; Seiffert et al. 2016).
332 In this context, it is very important to standardize protocols used in the characterization of
333 biodeteriorative (and bioprotective) processes, using approaches such as microscopic,
334 spectroscopic, culture-based, and ‘omics’ methods, in order to make scientific data truly comparable
335 and offer more reliable support for management decisions (Pinna 2014; Sterflinger et al. 2018).

336 Research on lithobiontic impacts on CHSS should also consider predicted impacts of climate
337 change on lithobiont colonization and growth (Davidson et al. 2018). Changes in temperature and
338 precipitation regimes, beyond their direct impact on CHSS, have been hypothesized to affect
339 lithobiont communities, causing potential shifts from biodeterioration to bioprotective impacts, and
340 vice versa (Viles and Cutler 2012; Fatorić & Seekamp 2017). As an example, Gómez-Bolea et al.

341 (2012) predicted that lithobiontic biomass on CHSS should increase in northern Europe whilst
342 decreasing in southern Europe because of contrasting trends in precipitation. Recent laboratory
343 experiments simulating changing water regimes and increased CO₂ concentrations have confirmed
344 the suggested impacts on biofilm composition and a shift towards biodeterioration effects (Prieto et
345 al. 2020). From a different point of view, subaerial biofilms have also been noted as potentially
346 important climate regulators, as agents of carbon sequestration, biogeochemical cycles and
347 elemental transformations (Villa et al. 2016).

348

349 **Approaches to managing lithobionts on CHSS**

350 *Factors affecting the opportunity to remove (or preserve) lithobionts*

351 Aspects of culture and tradition have shaped and continue to shape perceptions of the growth of
352 lithobionts on CHSS. Ruins covered by plants and other lithobionts, recognized as evidence of
353 natural decay, are a symbol of the European Romantic taste of the 19th century (Stanford 2000;
354 Huyssen 2006). Similarly, an abundance of lithobionts on some types of heritage sites, such as a
355 Celtic graveyard or a Shinto shrine, can be seen in a positive light today, especially in places where
356 local tradition tends to appreciate, rather than worry about, the merging of CHSS and nature,
357 sharing a common acceptance of human impermanence (Fitzsimons et al. 2012). In most cases,
358 however, alterations in CHSS caused by both biotic and abiotic drivers are considered to be
359 negative, reducing heritage values (Brimblecombe and Grossi 2005; Prieto et al. 2007). As a
360 consequence, removal of lithobionts is usually recognized as a necessary part of conservation
361 interventions (Pinna 2017). However, scientific evidence justifying the removal of lithobionts is
362 often missing.

363 In order to make more informed decisions about whether to remove (or preserve, or even encourage
364 further) lithobionts, aesthetic evaluations should be combined with data on the impacts of
365 lithobionts on stone durability, based on an analysis of the balance between biodeteriorative and

366 bioprotective processes. Such an approach is not in conflict with the priority often given (at least in
367 some countries) to aesthetic evaluations as the basis of management strategies for heritage sites, but
368 enables a broader, more balanced assessments of risks vs benefits. By way of an example, in cases
369 where lithobionts are shown to be predominantly bioprotective, their removal might cause a
370 decrease in durability of CHSS: if the aesthetic damage they cause is more severe, however, and
371 they are removed, strategies to substitute their bioprotective function should be considered. In cases
372 where the aesthetic damage caused by lithobionts is minor, and there is evidence of a bioprotective
373 effect, or of only minor biodeterioration impact, a recommendation could be made to preserve the
374 lithobionts and promote their biodiversity as an additional cultural value of the heritage site (Nimis
375 et al. 1992; Steinbauer et al. 2013). Such an approach may be particularly valuable in the case of
376 CHSS immersed in the natural environment, where the removal of a mature-climactic lithobiontic
377 community, similar to that found on surrounding natural outcrops, is often followed by a rapid
378 recolonization by a simplified community of ‘banal’ species (Nascimbene et al. 2009). The
379 inclusion of biodiversity in the concept of cultural value of a heritage site agrees with official
380 measures addressing parallel conservation of cultural and natural heritage (e.g. Italian Ministry for
381 Cultural Heritage and Activities 2004: the Italian code of cultural heritage and landscape; UNESCO
382 2014: Florence Declaration). This may be particularly important for cemeteries, monumental or
383 archaeological sites which are hotspots of lithology or microhabitat diversity and consequently host
384 strongly heterogeneous lithobiontic and plant communities, making them easily accessible to the
385 public (e.g. Nimis et al. 1987; Cicinelli et al. 2018; Löki et al. 2019). In other cases, cultural
386 heritage sites have been clearly identified as preferential sites for the preservation of threatened
387 species or even their re-introduction (Valkó et al. 2018; BLS 2020).

388 CHSS management should consider all facets of lithobiontic growths before automatically
389 removing lithobionts to conserve and present heritage sites. In all cases, once the decision has been
390 taken to remove lithobionts, the adoption of an effective strategy to clean the CHSS is an absolute
391 priority (Pinna 2017). A major issue for CHSS management is indeed the frequent need to repeat

392 treatments to control lithobionts, which in most cases are stress-tolerant (micro-)organisms well-
393 equipped to thwart human attempts to remove them and prevent their regrowth.

394

395 *Direct and indirect strategies to control lithobionts*

396 Two main strategies to control lithobionts on CHSS can be distinguished, although their choice
397 should not be considered exclusive and they may be used in combination: first, an indirect approach
398 focused on the control of microenvironmental parameters which favour/allow their growth, and
399 second, direct intervention to remove them from CHSS by physical and/or mechanical and/or
400 chemical methods (Pinna 2017). The first approach is based on the fact that the presence of
401 lithobiontic communities, and, in particular, of certain biodeteriogenic species, depends on the
402 availability of suitable microenvironmental conditions, allowing their establishment, expansion, and
403 reproduction. At any one heritage site, different CHSS often represent diverse ecological micro-
404 niches, hosting different lithobiontic communities which interact with the substrate and influence its
405 conservation in different ways (Marques et al. 2016; Tonon et al. 2019). Knowledge and monitoring
406 of (micro-)environmental parameters favouring or discouraging lithobiontic communities, and in
407 particular, deteriogenic species, can help recognise and achieve the best conservation solutions
408 (Caneva et al. 2016; Schumacher and Gorbushina 2020). For example, the development of
409 phototrophic biofilms on hypogean CHSS, and even on outdoor surfaces lit by artificial lighting,
410 may be controlled through careful illumination strategies, based on modifying the spectral emission
411 of lamps to affect microbial biomass, colour and EPS production (Albertano and Bruno 2003;
412 Sanmartín et al. 2017). Careful management of higher plants in heritage sites may limit their
413 shading of CHSS, with the consequent increase in humidity, and their supply of nutrients, both of
414 which favour lithobiontic growth (Salvadori and Charola 2011). Such ecologically-based
415 approaches may also be used to choose the most appropriate contemporary building materials
416 (Caneva et al. 2008).

417 Direct intervention to clean CHSS should satisfy the main aims of removing the lithobiontic
418 communities and preventing (re-)colonization processes by limiting surface bioreceptivity. These
419 objectives have been variously pursued by combining several mechanical, physical and chemical
420 approaches, already reviewed in detail elsewhere (Pinna 2017). The crucial point is that lithobiontic
421 colonization usually extends well beneath the rock surface and microbial structures generally show
422 a strong capacity to recover from disturbance, and so many cleaning interventions without
423 subsequent biocide treatment are usually followed by rapid recolonization (Sohrabi et al. 2016).
424 Removal by (pressurized) water and brushing may be effective in the case of superficial, subaerial
425 biofilms (Sanmartín et al. 2020), but in other situations they may spread microbial structures,
426 pushing them deeper within the substrate (Pinna 2017). Valuable protocols for the physical removal
427 of lithobionts by laser ablation have been drawn up in the last decade (Sanz et al. 2017), but even
428 the combination of scalpel and laser may sometimes fail to eliminate lithobiontic structures within
429 fissures (Rivas et al. 2018). Similarly, some laser ablation can cause damage to the mineral surface
430 whilst not completely removing the lichens (Pozo-Antonio et al. 2019). As a result of these issues,
431 biocide treatments often precede mechanical removal (Kakakhel et al. 2019). Although many of the
432 more toxic biocides have been banned, safer compounds may have some drawbacks. For example,
433 the effectiveness of quaternary ammonium salts (e.g benzalkonium chloride) to disrupt cell
434 membranes and kill lithobionts has been well demonstrated (Wessels and Ingmer 2013; Vannini et
435 al. 2018; Sanmartín et al. 2020), but their use can promote bacterial adaptation and antibiotic
436 resistance (Kampf 2018; Kim et al. 2018; Poursat et al. 2019). Moreover, their degradation is
437 suspected to contribute to nitrogen supply (Scheerer et al. 2009), thus favouring, rather than
438 preventing, recolonization by aggressive nitrophytic species. Such alarming findings call for caution
439 in biocide application (Stupar et al. 2014) and the need for non-chemical alternatives. As mentioned
440 above, electromagnetic methods, which include laser irradiation as well as UV, microwave and
441 gamma rays (Riminesi and Olmi 2016), are useful but still have limitations for large-scale
442 applications to outdoor CHSS. Alternatively, heat shock treatments have been shown to provide a

sustainable approach to killing lithobionts such as lichens and mosses (Tretiach et al. 2012; Bertuzzi et al. 2013). These poikilohydric organisms tolerate thermal stress well whilst dehydrated, but do not survive heating to 40-60°C for 6 hours when hydrated. Unfortunately, such an approach was less effective against microalgae (Bertuzzi et al. 2017) and the original proposal to exploit solar irradiation to provide the heating may be only suitable under stable weather conditions in warm countries, which limits its applicability. Nevertheless, the combination and improvement of all the cited and other approaches may be expected to shortly produce valuable strategies to moderate the use of biocides.

Whatever the chosen approach to kill lithobionts, it is necessary to monitor its efficacy on the target organisms and the examined CHSS: widely used biocides applied to lichens showed species- and site-specific effectiveness, likely depending on differences in the resistance of symbionts and the influence of different stone surfaces and climate/meteorological conditions (Favero-Longo et al. 2017). Biocides may have different effectiveness against epilithic and endolithic lithobionts (de los Ríos et al. 2012). Moreover, the method chosen to apply biocides, often defined for economic reasons, may strongly influence the final treatment success (Favero-Longo et al. 2017; Matteucci et al. 2019). A preliminary assessment of the viability of targeted microorganisms before and after treatment should be routinely performed by in situ methods, such as fluorimetry (Tretiach et al. 2010).

Although the effective devitalization of lithobionts contributes to keeping CHSS in a “clean” state for longer, (re-)colonization is inevitable (Fig. 3). However, longer term cleaning can be achieved by avoiding the application of compounds after cleaning which may increase bioreceptivity and promote recolonization, as demonstrated in the case of certain consolidants and other restoration products (Barriuso et al. 2017; Favero-Longo et al. 2018). In contrast, some other lithobiont control strategies may contribute to delay recolonization, including the application of photocatalytic nanoparticles (Fonseca et al. 2010; Sierra-Fernandez et al. 2017), and eco-friendly products, as essential oils and other biological substances exerting allelopathic effects (Fidanza and Caneva

2019). Monitoring programs are increasingly needed to monitor the long-term effectiveness of such approaches, in comparison with the traditional application of biocides following mechanical removal (Bruno et al. 2019; Favero-Longo et al. 2019; Sanmartín et al. 2020). Under certain climate conditions, however, even the most effective strategies may not fully prevent recolonization (Pinna et al. 2018), confirming the necessity of an integrated, site-specific approach (including microclimate control, where possible) to improve the success of management strategies for the conservation of CHSS.

Concluding remarks

Advances in technology and medical research have provided great tools to diagnose, control and prevent human diseases, and there is a clear integration between scientific research and managing health. This review has outlined that similarly integrated scientific approaches are available to diagnose the role of lithobionts on CHSS and inform management strategies. Lithobiont-CHSS systems display remarkable complexity, encompassing equilibria between lithosphere (i.e. the stone substrate) and biosphere (i.e. the lithobionts), as well as atmosphere/hydrosphere and anthroposphere, which are additional components affecting colonization and weathering dynamics. As in the case of human health, management decisions about the conservation of stone cultural heritage should increasingly take into consideration all the levels of knowledge available (or implementable) to decipher this complexity, and should be based on accurate diagnosis of each situation. Figure 4 presents a framework to include the above-reviewed spheres of investigation required for decision-making on lithobionts and CHSS, with a focus on whether to remove or preserve lithobionts and, if removal is chosen how to choose a suitable control strategy. As Figure 4 demonstrates, there is no ‘one size fits all’ approach because durability of different CHSS depends on the balance between biodeterioration and bioprotection effects driven by lithobiontic communities, which in turn depends on the species and lithology involved and the (micro)-environmental context. Moreover, these same factors (species-lithology-environment) also

determine the effectiveness of control strategies. Thus, whatever the protocol used (innovative or based on traditional techniques) an experimental assessment of its suitability for the CHSS of interest is vital. Funding limitations may discourage scientific investigations on the role of lithobionts and the efficacy of control strategies, but such investigations are vital for cost-effective and environmentally-sustainable management of CHSS in future.

500

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932 **Table**

933 Table 1 - Biodeteriorative and bioprotective roles often reported (●) or hypothesized/debated (?) for
 934 various types of lithobiontic organisms. It is worth noting that each potential role has to be
 935 considered species-specific and cannot be generalized for all the members of each group.

				Higher plants	Bryophytes	Lichens	Microbial biofilms		
							Algae	Cyanobacteria	Microcolonial fungi
Biodeteriorative roles	During life	Biogeophysical	"Rooting"	●	●	●			●
			Wetting/ drying			●	●	●	
			Enhanced thermal stresses		?	●	●	●	●
		Biogeochemical	Biomining		●	●		●	
			Acid/ complex dissolution	●	●	●	●	●	?
		Aesthetic loss	Surface coverage/ soiling	●	●	●	●	●	●
	After death	Remnant biocrusts	Disfiguring patina	?	●	●	●	●	●
Bioprotective roles	During life	Shielding	Umbrella effect	●	●	●	?	?	
			Reduced thermal stress	●	●	●	●	?	
			From pollutants	●	?	?	?	?	
		Biogeochemical	Rock varnish & case hardening			●		●	●
			Biomining		●	●		●	
		Aesthetic/ biodiversity enhancement	'Greening' walls	●	●	●	●	●	
	After death	Remnant biocrusts	Protective patina	?	●	●	●	●	●

936

937

938 **Figure captions**

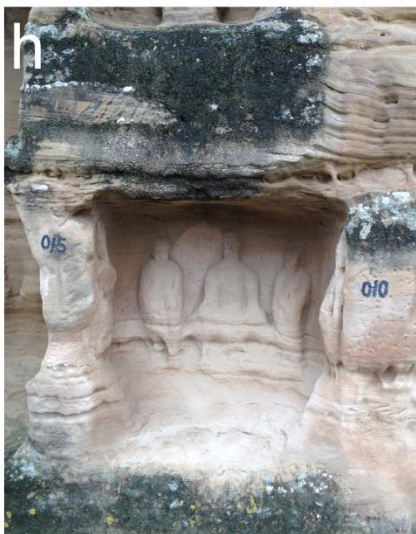
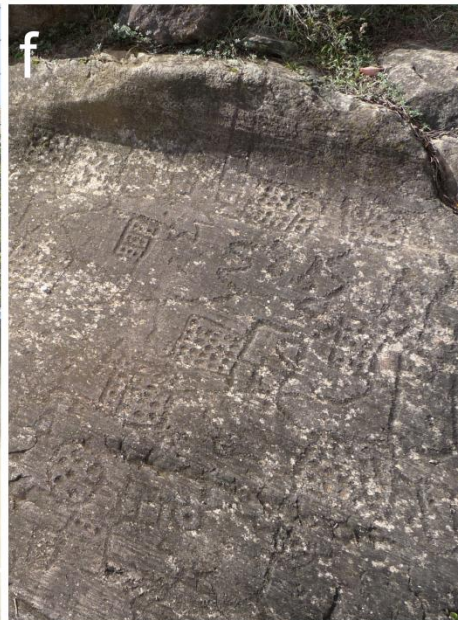
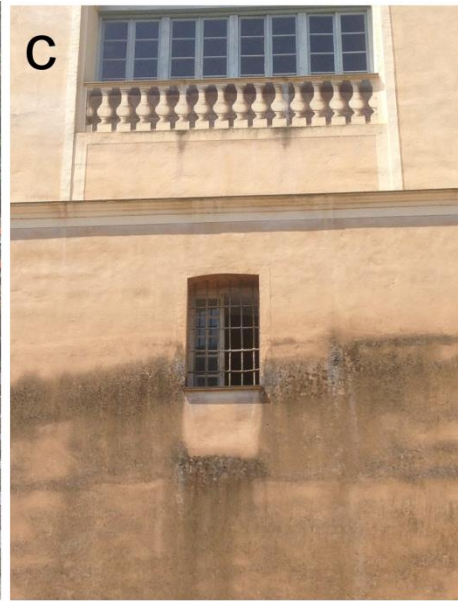
939 Fig. 1. Lithobionts on stone cultural heritage. **a** Residence of the Royal House of Savoy in Govone
940 (Italy; year 2010); **b** Fushimi Inari-Taisha-Shrine in Kyoto (Japan; 2019); **c** Sanctuaire de Notre-
941 Dame de Laghet in La Trinité (France; 2019); **d** Roman underground cistern down to Lithostrotos
942 in Jerusalem Old Town (2017); **e** 16th century Kelmscott Manor near Oxford (UK; 2018); **f**
943 Engravings of the ‘Rock of the Map’ in Valle Camonica (Italy; 2016); **g** St Andrew’s Churchyard in
944 the Isle of Portland (UK; 2018); **h** North Grotto Temple in the Gansu province (China; 2018); **i** The
945 Mostaccini Fountain in the Boboli Gardens of Florence (Italy; 2016).

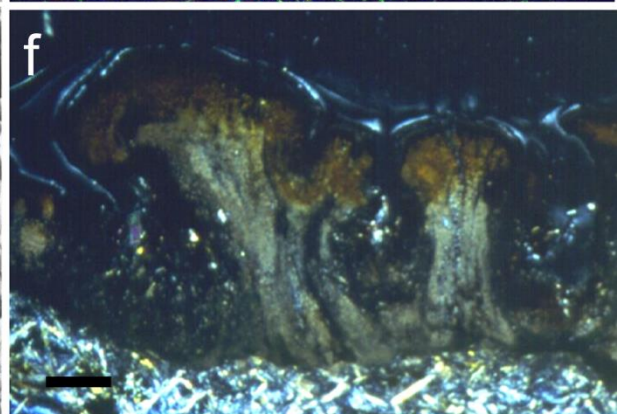
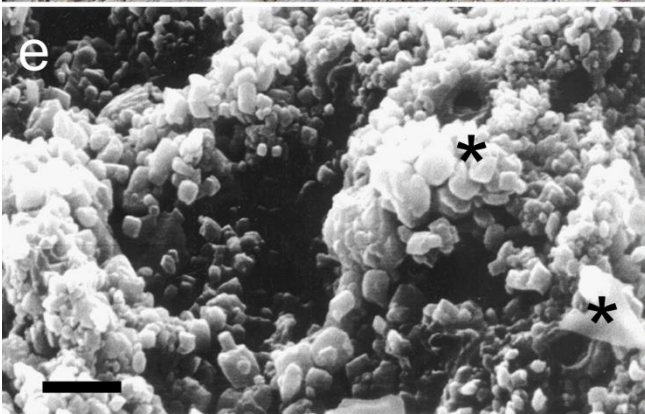
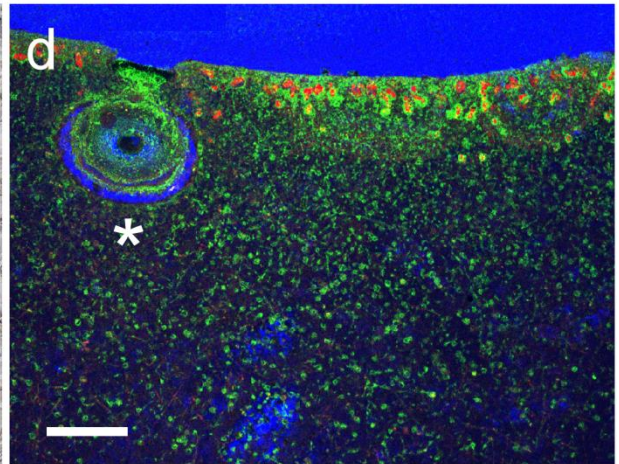
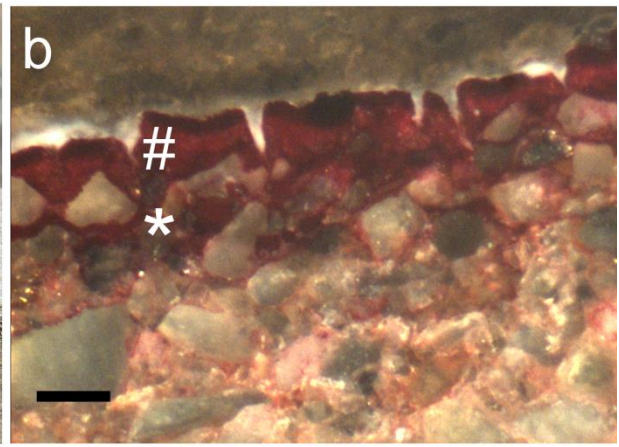
946 Fig. 2. Lithobiont-related processes and spatial patterning on CHSS: the example of lichen-forming
947 fungi. **a** rock flaking associated with lichen colonization of a sandstone (Pietra Serena) sculpted
948 surface. **b** Pietra Serena colonized by the lichen *Aspicilia cinerea* (L.) Körb., displaying the thalline
949 component (#) at the sandstone surface, and the hyphal penetration component (*) developing along
950 the grain borders (polished cross section stained by PAS and observed under reflected light
951 microscopy; bar: 500 µm); **c** pitting at the surface of a limestone previously colonized by an
952 endolithic lichen; **d** Aurisina limestone colonized by the endolithic lichen *Bagliettoa baldensis* (A.
953 Massal.) Vězda, displaying both the photobiont (red) and the mycobiont (green) developing within
954 the substrate, and a pit occupied by a fruiting body (*) (polished cross section stained with FITC-
955 Concanavalin A and observed under confocal laser scanning microscopy; bar: 500 µm); **e**
956 biomineralization of calcium oxalates at the interface between a lichen thallus and a serpentinite
957 rock (*, cross sectioned hyphae submerged by the oxalates; scanning electron microscopy; bar: 5
958 µm); **f** serpentinite colonized by the lichen *Lecidea atrobrunnea* (DC.) Schaer., displaying oxalates
959 (milk-like high birefringence colours) in the medulla layer and at the rock interface (thin cross
960 section observed under cross polarized transmitted light; bar: 500 µm).

961 Fig. 3. Colonization dynamics on CHSS. **a** lichens on a granite gravestone in the Walser graveyard
962 of the alpine village Gressoney-la-Trinité (Italy; year 2010), **b** the same surface after cleaning by
963 professional restorers (year 2011); **c** lichen recolonization after eight years (year 2019).

964 Fig. 4. Framework summarizing the spheres of knowledge dealing with the lithobiontic colonization
965 of CHSS (white boxes) and the potential control strategies (green), with the related diagnostic
966 approaches (blue). Such knowledge may properly support management decisions (orange) to
967 improve the conservation of stone cultural heritage.

968







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